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The Effects of Erythropoietin, Dibenzyline, and Iron Dextran on the Hemogram of Doe and Fetal Rabbits

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THE EFFECTS OF ERYTHROPOIETIN, DIBENZYLINE, AND IRON DEXTRAN ON THE HEMOGRAM OF DOE AND FETAL RABBITS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor — Date

Head, Entomology-Zoology Department — Date

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EAG

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INTRODUCTION

The iron deficiency anemia found particularly in swine has a high incidence in the newborn. The nutrition of the sow inversely affects progression of the anemia. Newborn nutritional status, also, inversely augments the anemic condition. The anemia does not become apparent in the newborn until a few days following parturition. There is a drastic decrease in iron availability with succeeding decreases in normal hemoglobin formation and mature red blood cell formation. An important factor along with these conditions is susceptibility to disease leading to death. A gradual return to normal follows if the newborn survives the anemic period. This condition is of economic significance to the swine producer for a variety of reasons.

The newborn piglet faces initially a fast growing period with a small reserve of storage iron for the developing tissues. The stored iron is quickly depleted and the anemia becomes apparent. The resulting erythropoietic malfunction may possibly be alleviated by prepartum maternal treatment and/or postpartum newborn treatment. Postpartum treatments may add additional stresses to the newborn enhancing the anemic condition.

Enhanced hemoglobin precursor transfer to the fetus may be of primary importance in carrying the newborn past the critical anemic period. The primary factor concerned with iron incorporation into the erythron and hemoglobin synthesis is the hormone, erythropoietin.

Several chemicals are being evaluated at the present time to provide an agent(s) that will successfully enhance hematological parameters of the newborn. A promising drug, dibenzyline, elicits serum iron level changes and increases peripheral blood flow.

The purpose of this study was to investigate selected hemogram parameters in pregnant rabbits and fetuses following prepartum treatments involving dibenzyline, injectable iron, and erythropoietin which may enhance hemogram parameters.

LITERATURE REVIEW

Regulation of Erythropoiesis

Oxygen

An important blood function is transporting oxygen to the tissue cells for metabolic processes. Hemoglobin, the conjugated protein, in the red blood cells is the primary means of oxygen transportation.

For some time it has been concluded that low oxygen partial pressure in the tissue cells elicits increased red blood cell production (erythropoietic effect) by the bone marrow. Dreyer and Walker[23] noted that at high altitudes where the oxygen tension was reduced there was an increase in the number of circulating erythrocytes. This has been substantiated by others.[58, 119, 123] Numerous researchers have demonstrated that hyperoxia and plethora (by transfusion of erythrocytes) created a suppression of erythropoiesis shown by a linear decrease in bone marrow activity.[13, 114, 135] These observations have led many researchers to subject animals to hypoxia (reduced atmospheric pressure) demonstrating subsequent increased red blood cell production.[5, 6, 29, 50, 124] Grant and Root,[59] however, could not demonstrate that regulation of erythropoiesis was directly dependent on the bone marrow oxygen concentration.

Hormonal Control of Erythropoiesis

Carnot and Deflandre[14] developed the original hypothesis that direct bone marrow oxygenation was not the controlling factor, but a humoral factor was the important substance that was produced.

in a direct response to hypoxia and released outside the bone marrow into the circulating blood. This hypothesis was developed through injection of anemic rabbit serum into normal rabbits. There were very few investigations concerning this aspect for many years following their experiments.

Within the last two decades many experiments have demonstrated a humoral factor in the blood. Reissman[112] showed corroborative evidence of the humoral factor utilizing parabiotic rats (artificial siamese twins) and subjecting one partner to chronic hypoxemia, the other to normal atmosphere. Significant evidence showed that the partial pressure of oxygen in bone marrow did not directly effect erythropoiesis. A humoral factor elicited by hypoxemia in one partner was transfused to the other.

Attempting to locate the oxygen sensitive organ which elaborated the humoral factor, Stohlman et al.[131] demonstrated the factor as being produced inferior to the diaphragm in humans. Krumdieck[74] in an early experiment studied the effects of anemic animal serum injections on peripheral blood and bone marrow of normal recipient animals. Erslev[24] demonstrated the plasma factor stimulated bone marrow activity because red blood cell production increased.

The plasma factor was labeled hemopoietine by Carnot and Deflandre.[14] This was later changed to the more widely accepted erythropoietin by Bonsdorff and Jalavisto[8] because it was believed to control primarily red blood cell production.

Erythropoietin Production

Attempts to locate the site of erythropoietin production have led to excision of many organs and to organ extractions for the analysis of the hormone. Gordon has led the field in this early work. Gordon et al.[53] compared plasma, blood cells, liver, and bone marrow from anemic rabbits to normal rabbits for erythropoietic activity. Reticulocytosis, erythrocytosis, increases in hemoglobin, and packed cell volume were noted in extracts of blood cells, liver, and plasma. Gordon et al.[52] expanded his previous research using extracts of spleen, thymus, lung, brain, skin, pancreas, skeletal muscle, bone marrow, plasma, and packed blood cells of anemic rabbits. When tested in normal rats, only plasma showed erythropoietic activity.

Jacobson et al.[66] and Erslev[25] demonstrated the liver did not produce erythropoietin, contrary to the results of Mirand et al.[94] and Lange and Gallagher.[78] An increase in erythropoiesis failed to occur in rats exposed to reduced oxygen pressures when their livers were damaged with carbon tetrachloride.[94] Gordon[48] summarized the literature in that plasma or serum constituted the most concentrated source of erythropoietin in a hypoxic animal.

Kidney

Jacobson et al.[66] first suggested the kidney was the site of erythropoietic stimulating factor (erythropoietin) production. Bilateral nephrectomized rats and rabbits demonstrated no erythropoietic response to cobalt administration with increased levels of plasma erythropoietin. Bilateral nephrectomized dogs have also demonstrated

abolished erythropoiesis, by decreased Fe^{59} incorporation into circulating red blood cells; almost total erythroid aplasia.[68, 97, 98]

Naets[98] raised the question whether the decrease of iron incorporation was due to toxic product retention or actual absence of the renal factor. Nephrectomized animals showed lower erythropoietic rates, as measured by Fe^{59} uptake, compared to ureter altered animals such as, ureter ligation in dogs and rats,[46, 99] ureter section in rats,[99] and ureterovenous anastomosis in dogs.[132]

In parabiotic rats subjected to hypoxia, Fe^{59} incorporation was less in nephrectomized partner than in the ureter ligated partner.[116] Iron incorporation in the pairs where the nephrectomized partner was hypoxic was greater than in those pairs where one was nephrectomized but neither hypoxic. This suggested other tissues than kidney contributed to the erythropoietic response. Gallagher et al.[41] favored this conclusion in rabbits when hypoxic nephrectomized rabbit plasma extracts increased Fe^{59} incorporation 9.5% in polycythemic rats while normal plasma extracts and nonstimulated nephrectomized rabbit plasma extracts increased rat Fe^{59} incorporation 5.7% and 5.0% respectively.

Nathan et al.[101] using four renaprival patients (hemoglobin conc.—7-9 gm./100 ml.) showed an erythropoietic response to hypoxia in two patients and concluded there was other than a renal source of erythropoietin for red blood cell production. Abbrecht and Malvin[1]

and Hansen[63] came to the same conclusion using hypoxic dogs and measuring differences in the titer of plasma erythropoietin between the renal artery and the renal vein by Fe^{59} uptake. They showed no difference between uptake. Possibly, the amount of erythropoietin was so small that it could not be detected in the analysis.[2]

Renal hypoxia initiated by the use of the Goldblatt clamp on the renal artery has afforded more of a physiological situation for demonstrating erythropoietin titer differences. Noteworthy studies by Fisher and Samuels[34] employed this method using varying degrees of constriction and showed an increase in plasma levels of erythropoietin at 12 hours up to a constriction of the artery at 57.5% to 71% of the preconstriction values. Takaku *et al.*[134] employing both nephrectomized rats and unilateral renal artery constricted rats demonstrated the need for the kidney in increasing plasma erythropoietin levels following blood loss.

Another major study avenue utilized was the isolated *in vitro* perfusion of the hypoxic kidney. Fisher and Birdwell,[32] Kuratowska *et al.*,[77] and Zangheri *et al.*[141] utilized the perfused kidney and observed erythropoietin in the renal vein blood. This was the most direct evidence that the kidney was the major, if not, the sole site of erythropoietin production.[77]

Erslev *et al.*[28] in a critical review of the *in vitro* perfusion technique suggested the conclusion that erythropoietin was produced by the kidney in response to hypoxia was not justified. He used dog lung kidney preparations and showed no generation of the erythropoietic

material after four hours of perfusion with normal, hypoxic, or anemic blood. He stated that erythropoietin was released only from injured and disintegrating tissue and not from metabolically active, well preserved kidneys.

Pavlovic-Kentera et al.[106] defined the situation further using perfused kidneys of dogs. In nine experiments only 50% of the animals showed significant increases in erythropoietin levels in the renal vein after five hours of perfusion, while all animals showed significant increases in erythropoietin titer after longer periods of time.

The presence of high serum levels of erythropoietin associated with erythrocytosis has been found in patients with renal neoplasms and cysts but this was indirect evidence of renal erythropoietin production since nonrenal neoplasms are described with high erythropoietin serum levels.[140]

To further the importance of the kidney in erythropoietin production a quantitative assay measure of erythropoietin activity needs to be developed.[3, 126] This may prove that erythropoietin is physiological in emergencies but under normal conditions is involved mainly in fine red blood cell adjustments.

Nature of Erythropoietic Factor

It has been suggested for a decade that two humoral factors regulated control of erythropoiesis. One, being heat stable and ether soluble which stimulated cellular division in erythroblasts. The other being heat labile and ether insoluble which augmented hemoglobin

synthesis.[82]

Kuratowska et al.[76] elaborated on several theories to explain the two factors. The renal factor has been demonstrated in the perfusate of the isolated kidney. Incubation of it with plasma (required alpha globulin) yielded an erythropoietically active substance.[15, 43] The theories debated are as follows: 1) The renal factor, REF (renal erythropoietic factor), may be a precursor of erythropoietin and be activated by a system in the alpha globulin portion of blood.[76] 2) The renal factor may act as an enzyme, and activate a precursor in the alpha globulins to yield erythropoietin.[49, 70, 142] Stohlman[128] noted the similarities of this mechanism with the renin-angiotensin complex. 3) The renal factor may combine with a portion of the alpha globulin to heat stabilize and activate it.[15, 49, 75]

Study of the above theories was complicated by the rapid turnover rate of erythropoietin, possibly due to accumulation of an inhibitor.[113] Dalos et al.[19] using X-ray irradiation of rabbit spleen isolated a factor that decreased heme synthesis in red blood cells. Kuratowska[75] theorized an erythropoietin stimulator and inhibitor that controled the erythropoietic system.

Kidney Production Site of Erythropoietin

Osnes[105] suggested the renal cortex, mainly the juxtaglomerular apparatus, as the site of erythropoietin production. Abbrecht et al.[2] and Muirhead et al.[97] noted significantly higher levels of erythropoietin released from the renal cortex than other areas. The juxtaglomerular cell under anemic and high altitude conditions have

greater secretory granules and elaborate large amounts of erythropoietin.[21, 64]

Tobian[136] noted the relationship between the abundance of granules in the juxtaglomerular cells and the arterial pressure together with electrolyte concentrations, an inverse relationship. This granularity was correlated with large quantities of extractable renin. Renin and erythropoietin were demonstrated to be separate substances.[2] Goldfarb and Tobian[44] in hypoxic and hyperoxic experiments showed no change in juxtaglomerular cell granularity after an extended period of time. They concluded hypovolemia rather than hypoxia elicited the response in cell granularity.

Baum et al.[7] and Fisher et al.[35] using rabbit anti-sheep erythropoietin antibodies, found the glomerular tuft as the site of erythropoietin elaboration in anemic sheep kidneys with no staining in the juxtaglomerular area or in the spleen, lymph nodes, or liver. Wesson[140] stated that no more than indirect evidence has been offered to establish the cellular source of renal erythropoietin.

Properties of Erythropoietin

There existed many conflicting viewpoints on the chemical and physical nature of erythropoietin. The following list was complied from various literature.

1. Erythropoietin was stable to heat at pH 5.5-9.0, and destroyed at pH 1 or 13.[81, 111, 122]

2. Resisted breakdown at 100° C. temperature for a few minutes.[45]

3. Digested by pepsin, chymotrypsin, trypsin,[54, 122] and

sialidase.[45, 76]

4. Using 200 fold purification of erythropoietin by Dowex 50 chromatography, erythropoietin was found to be a glycoprotein—36% protein, 15% carbohydrate, 3.7% sialic acid, and 1.1% hexosamine.[45, 76]

5. Continuous flow electrophoresis has shown it to migrate as an alpha-2-globulin.[111]

6. Erthropoietin has a probable molecular weight between 60,000 and 70,000,[45] while a molecular weight of 27,000 has been observed.[115]

Mode of Action of Erythropoietin

Erythropoietin levels in the plasma are primarily influenced by the state of the erythroid tissue[125] and relationship between oxygen supply and demand.[39, 125] When oxygen demand became severe the response to erythropoietin was limited primarily by the oxygen requirement for oxidative metabolism in red blood cell precursors, which was necessary for maximum response to erythropoietin.[129]

Early work by Krumdieck[74] utilizing rabbits demonstrated significant changes in circulatory reticulocytes three days post-injection of anemic serum but no changes in circulation red blood cells or hemoglobin at that time. Filmanowicz and Gurney[30] noted in mice following administration of erythropoietin that after three days peripheral reticulocytosis appeared. The wave of erythropoiesis also showed at 24 hours a peak in proerythroblasts and at 72 hours a peak in normoblasts. He concluded that the stem cell was the site of action for erythropoietin. Later observations were consistent with erythropoietin action being directed toward differentiation of stem

cells.[27, 140] Using rabbit antihuman erythropoietin antibodies in mice with tritiated thymidine (detect increase in mitotic activity), Schooley and Garcia[120] observed an erythropoietin effect on differentiation of stem cells with no effect on maturation of erythroid cells. Conversely, Gallagher et al.[42] concluded that erythropoietin exerted its effect on differentiation of erythroid cells into mature erythrocytes. Borsook et al.[9] found, measuring C^{14} incorporation into hemoglobin, when an erythropoietic crisis was present cells by pass the orthochromatic erythroblast stage in maturation to shorten the maturation time.

Stohlman[127] supported a single mode of action involving changes in differentiation rates of stem cells and/or erythroid cells by erythropoietin. This theory involved erythropoietin interaction with an unknown enzyme to form a product capable of initiation of hemoglobin synthesis. Others shared similar conclusions in supporting erythropoietin enhancement of hemoglobin synthesis in differentiated erythroid cells.[73] Stohlman et al.[130] defined an intermediate precursor cell, a more differentiated stem cell, as being affected by erythropoietin.

Recent research suggested erythropoietin activation of stem cells was closely associated with DNA synthesis.[104] Krantz and Goldwasser[72] demonstrated an activation of messenger RNA by erythropoietin which caused an initiation of the metabolic chain reaction leading to formation of mature red blood cells.

Other Factors Influencing Erythropoiesis

The presence of an extrarenal erythropoietic factor has been observed by researchers. Bilateral nephrectomy in rabbits[26, 38] and excision of the kidneys in rats[93] demonstrated the preservation of some erythropoietic activity. Antiserum of rat renal erythropoietin neutralized the plasma erythropoietic factor elaborated in nephrectomized animals.[38] The search for an extrarenal erythropoietin source lead researchers to different organs.

Central Nervous System

The central nervous system (CNS) has been studied for its effect on erythropoiesis. The hypothalamus was indicated as exerting a regulatory function[121] while the caudate nucleus, hippocampus, and cerebrum had no function.[87]

Electrical stimulation of the hypothalamus in rabbits,[121] and monkeys[92] augmented erythropoiesis by increasing Fe^{59} incorporation into erythrocytes and reticulocytes, and increasing blood volume. Conversely, Piliero et al.[109] in rats and Mirand et al.[92] in monkeys with hypothalamic lesions found no change in the erythropoietic response.

Mirand[89] postulated the effect was due to a nervous pathway and/or hormone on the erythropoietin producing organ(s). Medado et al.[87] further proposed the autonomic nervous system (ANS) pathway to the bone marrow as the means of action. He demonstrated this by suppression of the erythropoietic effect following atropine administration prior to stimulation. Halvorsen et al.[62] suggested an

effect through the pituitary gland release of hormones and/or ANS on blood flow in erythropoietin producing organs or bone marrow.

Endocrine

Hypophysectomy reduced erythropoiesis greatly within one to three weeks in rats, but the ability to produce erythropoietin was still present.[67] The role of the pituitary as a primary effector of erythropoiesis has been refuted.[40, 61, 107] Crafts and Meineke[16] suggested a secondary role of the pituitary in erythropoiesis through thyrotrophic hormone, adrenocorticotrophic hormone and growth hormone effects on general metabolic changes leading to decreased oxygen demand and eventually decreased erythropoiesis.

Testosterone administration has stimulated erythropoiesis in polycythemic mice.[36] Several investigators suggested testosterone promoted erythropoiesis by stimulating the elaboration of erythropoietin. Fried and Gurney[37] stated that kidney growth was stimulated by testosterone; especially epitheleal cell cytoplasm. Conversely, Meineke and Crafts[88] suggested that testosterone acted synergistically with hypoxia or erythropoietin to exert its erythropoietic effects. Mirand et al.[91] supported synergism between hypoxia and androgens in activating adrenocorticotrophic hormone release leading to increased corticosteroid release. Naets and Wittek[100] presented evidence supporting the concept that testosterone potentiated the action of erythropoietin by either increasing stem cell sensitivity to erythropoietin or increasing actual numbers of stem cells.

Estrogen inhibited erythropoiesis probably by suppressed pro-

duction or activity of an extrarenal precursor erythropoietin.[55, 90] During prolonged administration of a massive dose of estrogen in rats erythropoietin production was depressed and iron utilization was impaired because it was fixed in iron laden cells.[108] Gordon et al.[51] supported direct action of estrogen on erythropoietic tissue to diminish the number or response of stem cells to erythropoietin. Conversely, Jepson and Lowenstein[69] noting high plasma estrogen levels and correspondingly high erythropoietic activity in pregnant mice suggested inhibition of Fe^{59} incorporation into stem cells but not later erythroid cells.

Cobalt

Cobalt, a metal capable of stimulating erythropoiesis, has its major effect on erythropoietin production.[47] The inactivation of sheep erythropoietin in rat kidney homogenates has been prevented by adding high concentrations of cobalt.[33] Erythropoietin recovery was greater in the cobalt homogenates than in the non cobalt homogenates. This lead to the theory of cobalt acting as an antagonist to a renal erythropoietic inhibitory factor.

Kuratowska[75] noted the effects of CNS (hypothalamus), hormones (androgens, growth hormone, and others), oxygen supply, red blood cell mass, and other factors (cobalt) on the production of erythropoietin by the kidney. He stated 10% of the erythropoietic factor was produced extrarenally in normal humans.

Fetal Erythropoiesis

Transfer and Uptake of Iron by the Fetus

Through the use of Fe^{59} the plasma has been found to be the important source of fetal iron, not red blood cell hemoglobin.(110, 138) Morgan(95) demonstrated in pregnant rats and rabbits a decrease in hemoglobin and plasma iron towards parturition with an increase in fetal utilization of iron. A marked increase in radioiron uptake by the placenta and fetus with advancing gestation, with a sharp increase during the last trimester, has been found in rabbits(20) and swine.(65)

Hoskins and Hansard(65) in sheep noted three stages of fetal hemopoiesis; in liver and spleen early in fetal life, in red bone marrow in the second trimester, and equally in all tissues in the last trimester. Incorporation into developing tissue seemed to have the greatest influence on transfer rate of iron. Also implicated in the regulation of iron transfer were the general state of maternal iron metabolism and the iron supply in the maternal plasma.(10) Placental transfer of iron occurred against a concentration gradient with uptake by the placenta being an active process while retrograde transfer back to maternal tissue did not occur.(10)

Ferritin-Transferin Transport Mechanism

Granick(56) demonstrated the presence of ferritin in the intestinal mucosa of guinea pigs. By feeding iron to these animals he noted an increase in the concentration of apoferritin, appearing in the form of ferritin, which regulated the absorption of iron. Rush et al.(117) proposed when ferritin was present in high concentration in the mucosal cells active transport of iron was inhibited through the cells. This mechanism is the subject of lively disputes. In iron deficiency situations a lack of ferritin in the mucosal cells

allowed free passage of dietary iron into the cells and thence into the body.[17] Late in pregnancy iron transport across the gut increased with *in vitro* gut sacs and *in vivo* duodenal loops.

A two-step mechanism for iron absorption has been suggested.[12, 18, 84] First, rapid mucosal uptake of iron occurred in a non-protein iron form. Following this ferritin (protein-bound iron) formation occurred in the cell. Second, slow serosal transfer of iron to the blood, a rate limiting step, remained constant and maximal. This second step was more dependent on oxidative metabolism.

Nylander[103] observed the presence of ferritin in the allantoic placenta of normal rats but in the anemic group little ferritin was found. Fineberg and Greenberg[31] measured the incorporation of C^{14} from labeled amino acids into liver ferritin and demonstrated that administered iron accelerated *de novo* synthesis of the protein portion of ferritin. Granick[57] reviewed this theory stating an increase in apoferritin, generally parallels an increase in iron.

Transferrin, plasma iron-binding protein, bound by the placenta released its iron to rat placental tissue.[79, 80] Larkin *et al.*[79] demonstrated in pregnant rabbits a release of iron from transferrin to the maternal circulation.

Transferrin from maternal serum transfered to fetal serum in rats and rabbits while some was bound to placental tissues.[96] Masters *et al.*[85] also demonstrated transferrin transmission to the fetus in rats. Transfer was low until the last three days of gestation when it increased rapidly.

Transfer of Erythropoietin

Jacobson and associates[68], using hypertransfusion in pregnant rats, suppressed maternal but not fetal erythropoiesis. They concluded that fetal erythropoiesis operated independent of maternal erythropoiesis. They offered two theories: 1) the fetus produced erythropoietin, therefore erythropoietin did not cross the placenta to maternal tissue, or 2) fetal erythropoiesis operated independently of the erythropoietic mechanism. In favor of the latter theory was that erythropoiesis developed before the functionally intact kidney. Zanjani et al.[143] found fetal erythropoietin production induced by hypoxia with the amount of erythropoietin being proportional to the intensity of the anemia and no maternal erythropoietin influencing the fetus.

Iron Relationships

McGowan and Crichton[86] first diagnosed nutritional iron deficiency anemia in suckling pigs. Since that time administration of various chemicals in combination with iron have been utilized to remedy this condition.

Iron dextran given during the suckling period in baby pigs has proven successful in maintaining normal hemoglobin values.[137, 144] Also, iron dextran given two weeks prepartum to the sow maintained high hemoglobin levels during the critical postpartum period in the newborn. Other preparations, including peptonized iron,[139] ferric ammonium citrate,[22] ferric phosphate, and iron copper paste have produced generally inferior results.

Synergistic roles of iron with various compounds have been

utilized to enhance placenta transfer of iron with resulting alleviation of the neonatal anemia. Swanson[133] investigated partial protein-free filtrate (PPFF), iron dextran, and B vitamin effects in swine. Prepartum sow and postpartum piglet treatment were compared to saline controls. Hemogram parameters showed iron dextran postpartum alleviated the neonatal pig anemia. Combined PPFF (erythropoietin) and iron dextran prepartum and postpartum produced an erythron response. PPFF seemed to be synergistic in placental transfer of iron. All other combinations showed little or no significant hemogram changes. Ulbrey et al.[137] administered iron dextran and B vitamins postpartum to piglets and found no hematological improvement over controls.

Lippert[83] investigated the synergistic effects of iron dextran, rabbit PPFF, and vitamin C administered prepartum in rabbits. He found no enhanced transfer of iron or erythropoietin to the fetus.

Dibenzyline (N-phenoxyisopropyl-N-benzyl-β-chloroethylamine hydrochloride)

Schade et al.[118] administered phenoxybenzamine (dibenzyline) to rats prior to oral dosage of Fe^{59}. He demonstrated increased serum iron levels and increased plasma iron clearance following administration.

Dibenzyline is a representative type of an alpha adrenergic blocking agent. Dibenzyline has a slow onset of action even after IV administration, due to the time required for formation of reactive intermediates.[102] The methyl side chain group increased absorbability in the intestinal mucosa.

Pharmacologically dibenzyline produced longer acting blockage than any other adrenergic blocker.[71] Cardiovascular effects are varied in normotensive individuals including increased cardiac output, decreased total peripheral resistance, and induced fall in blood pressure.

Dibenzyline was first used clinically in 1950.[60] Dibenzyline was effectively administered by all routes. It has a high solubility at body pH with accumulation in fat at large doses. Agarwal and Harvey[4] noted prolonged action due to slow release from a receptor substance in the tissue. Brodie et al.[11] observed metabolization of dibenzyline by dealkyation to the corresponding amine then excretion by the kidneys.

MATERIALS AND METHODS

Experimental Animals

Twenty-four Dutch Belted virgin doe rabbits weighing between 1500 and 1900 grams were utilized for this investigation. All rabbits were examined initially and found to be alert, active, and apparently in good health. Experimental animals were individually caged in stainless steel batteries and maintained on a commercial rabbit pelletized diet[1] and water ad libitum.

General Procedures

Animals were adapted for 10 days to the experimental environment. A table of random numbers was used to separate the does into experimental and control groups, each containing three animals. Does were mated to Dutch Belted males twice on the same day.

Pretreatment blood samples of 5 ml. were taken via cardiac puncture on day 25 of gestation using aseptic techniques and employing sodium heparin as an anticoagulant. Cardiac puncture preparation of the unanesthetized rabbits involved thoracic shaving and applying Zephiran Chloride diluted 1:750 topically.

Doe treatments were administered on day 25 of gestation as follows:

Group I - saline controls (C)

Group II - iron dextran (Fe)

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[1]Rabbit Chow, Ralston Purina Company, Checkerboard Square, St. Louis, Missouri 63188.

Group III - erythropoietin (Ep)

Group IV - dibenzyline (D)

Group V - iron dextran and erythropoietin (Fe+Ep)

Group VI - dibenzyline and erythropoietin (D+Ep)

Group VII - iron dextran and dibenzyline (Fe+D)

Group VIII - iron dextran, dibenzyline, and erythropoietin (Fe+D+Ep)

Treatments were as follows for the different groups: three ml. of saline were administered intraperitoneally to control animals; fifty mg./kg. of iron dextran[2] were administered intramuscularly; two units/kg. of erythropoietin[3] and saline were administered intraperitoneally; and twelve mg./kg. of dibenzyline[4] and saline were given intraperitoneally.

Posttreatment doe blood samples were taken on day 30 of gestation via cardiac puncture in the same manner as pretreatment samples. Does were sacrificed on day 30 by inducing air embolism and the fetuses were removed by cesarean section. The fetuses were decapitated and adequate blood samples taken for duplicate red blood cell counts, packed cell volumes, and hemoglobin determinations.

Analytical Procedures

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[2]Pigdex Iron-Dextran Injection, Agricultural Division, American Cyanamid Company, Princeton, New Jersey 08540.

[3]Connaught Medical Research Laboratories, University of Toronto, Willowdale, Ontario Canada.

[4]Smith, Kline and French Laboratories, Philadelphia, Pennsylvania 19101.

Red Blood Cell Count

Red blood cell counts were obtained employing a Coulter Counter Model F.[5] The counter was standardized for rabbit red blood cells with the attenuation set at .707, aperture at 16, and threshold at 7. The dilution of 1 to 50,000 was accomplished by a two step method. First, 40 lambda of blood was added to 20 ml. of normal saline (0.90% sodium chloride and 0.1% sodium azide) utilizing a Coulter Counter Dual-Diluter.[6] Second, 200 lambda of the above solution was added to 20 ml. normal saline. The counts were performed in duplicates. The background counts on the saline solution were performed and considered insignificant if less than 200. The red blood cell count found was corrected with the Coulter Counter Coincidence Correction Chart[7] for two or more cells passing through the aperture at the same time.

Hemoglobin Concentration

Duplicate hemoglobin values were obtained by using the cyanmethemoglobin technique.[8] A Hycel Cuvette Chemistry System[9] was calibrated to deliver the appropriate amounts of Hycel[R] reagent

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[5]Coulter Electronics, Inc., Hialeah, Florida 33010.

[6]Ibid.

[7]Ibid.

[8]Hycel Cyanmethemoglobin Determinations, Hycel, Inc., Houston, Texas 77036.

[9]Hycel, Inc., Houston, Texas 77036.

(6.0 ml.). A sample volume of 0.02 ml. of blood was removed and added to the 6.0 ml. of reagent. This solution was read against a reagent blank at 540 millimicrons on a Coleman Junior II Spectrophotometer Model 6L20.[10] Readings were transferred to a standard curve and total hemoglobin concentration in gm./100 ml. obtained directly.

Packed Cell Volume

Packed cell volumes (PCV) were determined employing the micro-hematocrit method.[11] Duplicate blood samples were introduced into heparinized microhematocrit capillary tubes. The tubes were centrifuged for five minutes at 12,500 rpm in an Adams Micro-Hematocrit Centrifuge.[12] Values were obtained employing the Adams Micro-Hematocrit Tube Reader (Chart Type).[13]

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the following formulas:

$$\text{MCV (cu.)} = \frac{\text{packed cell volume} \times 10}{\text{erythrocytes, millions/cmm.}}$$

$$\text{MCH (uug.)} = \frac{\text{hemoglobin concentration, gm./100 ml. blood} \times 10}{\text{erythrocytes, millions/cmm.}}$$

$$\text{MCHC (\%)} = \frac{\text{hemoglobin concentration, gm./100 ml. blood} \times 100}{\text{packed cell volume}}$$

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[10] Coleman Instruments, Maywood, Illinois 60153.

[11] Adams Micro-Hematocrit Technique, Clay-Adams, Inc., New York, New York 10010.

[12] Clay-Adams, Inc., New York, New York 10010.

[13] Ibid.

RESULTS

Individual hemogram values of does receiving prepartum treatments along with control animals are listed in the appendix in tables 5 through 12. Three does died during cardiac puncture and three does were non gravid. Mean pre and posttreatment doe hemogram values are listed in tables 1 and 2, respectively. Overall mean pretreatment hemogram values for the 23 experimental does were: 4.91×10^6 erythrocytes/cmm.; 11.4 gm. hemoglobin/100 ml. whole blood; 34.4% packed cell volume; 71.3 cμ., MCV; 23.5 μμg., MCH; and 33.1%, MCHC.

Individual doe hemogram values changed within groups although the amount of change was not significant in an analysis of variance. However, a lack of significance possibly should be expected because of the number of does used and doe to doe variability. Certain does showed notable trends. Does 2 and 3 in the iron dextran treated group demonstrated increased RBC count, Hb, and PCV with corresponding decreases in MCH values. Doe 3 had increases of about 120% for RBC count, about 80% for Hb concentration, and about a 20% decrease in MCH. Combining erythropoietin and iron dextran elicited increased RBC, Hb, and PCV values for all three does. Doe 3 values in the dibenzyline treated group decreased notably; about 30% for RBC count and 20% for Hb, while the MCH value increased. Iron dextran and dibenzyline treated does demonstrated increased RBC counts along with decreased MCV values. Treatment with a combination of erythropoietin, iron dextran, and dibenzyline decreased doe Hb values.

Mean doe values for treated groups changed notable following

treatment with the three compounds. Ditenzyline administration in group IV decreased all parameter values during the treatment period, especially the RBC count, Hb, and PCV about 15%, 20%, and 16.5%, respectively. Erythropoietin and iron administration increased RBC count, Hb, and PCV by about 25%, 14.6%, and 22% respectively.

Analysis of variance results on fetal data are summarized in table 4. Significant changes are noted in the table.

Mean fetal rabbit hemogram values are recorded in table 3, while individual values are listed in tables 5 through 12. Comparing mean fetal hemogram values of the experimental animals to the controls indicated dibenzyline treatment caused the greatest increase in RBC, Hb, PCV, and MCV values. Group VIII had the largest increase in the MCH and MCHC values over controls. Groups VIII and VI elicited similar responses as group IV in the MCV and MCH values. Combination treatments of dibenzyline and erythropoietin decreased RBC, Hb, and PCV values over controls; the only treatment that showed this alteration, though this group had high MCV and MCH values. Weight increases by each group over control fetuses were noted.

Table 1: Average Pretreatment Doe Rabbit Interactions of Iron Dextran, Dibenzyline, and Erythropoietin.

Source (treatment)	RBC (10^6/cmm.)	Hb (gm./100 ml.)	PCV (%)	MCV (cμ.)	MCH (μμg.)	MCHC (%)
No D, Ep, or Fe	5.20	12.30	38.15	73.44	23.66	32.26
D	5.32	12.33	36.87	69.23	23.16	33.45
Ep	4.25	10.73	32.17	78.93	25.45	33.33
D+Ep	5.38	11.73	35.30	65.69	21.88	33.27
Fe	4.74	10.87	32.27	72.51	24.12	33.27
D+Fe	5.04	11.90	36.33	72.24	23.67	32.70
Ep+Fe	3.93	9.57	27.67	70.55	24.78	35.23
D+Ep+Fe	5.42	11.70	36.83	68.07	21.49	31.59

Table 2: Average Posttreatment Doe Rabbit Interactions of Iron Dextran, Dibenzyline, and Erythropoietin.

Source (treatment)	RBC (10^6/cmm.)	Hb (gm./100 ml.)	PCV (%)	MCV (cμ.)	MCH (μμg.)	MCHC (%)
No D, Ep, or Fe	5.34	11.55	35.00	66.20	21.92	33.09
D	4.54	9.70	30.75	68.39	21.85	31.88
Ep	5.03	10.63	34.00	67.80	21.17	31.23
D+Ep	5.36	10.93	34.83	64.95	20.38	31.37
Fe	5.08	11.70	35.50	70.25	23.12	32.86
D+Fe	5.91	11.87	33.83	57.14	20.16	36.59
Ep+Fe	4.90	10.97	33.83	69.30	22.47	32.46
D+Ep+Fe	5.82	12.00	37.25	64.34	20.70	32.19

Table 3: Fetal Average Main Effects and Interactions of Iron Dextran, Dibenzyline, and Erythropoietin.

Source (treatment)	Fetal Weights (gm.)	RBC (10^6/cmm.)	Hb (gm./100 ml.)	PCV (%)	MCV (cμ.)	MCH (μμg.)	MCHC (%)
Dibenzyline (D)							
No D	35.37	3.63	11.84	44.70	123.81	32.72	26.53
D	32.98	3.54	12.21	45.28	128.64	34.69	27.00
Erythropoietin (Ep)							
No Ep	34.20	3.62	11.99	45.51	126.18	33.19	26.40
Ep	34.14	3.55	12.06	44.47	126.27	34.21	27.13
Iron Dextran (Fe)							
No Fe	33.74	3.56	11.76	44.80	126.73	33.20	26.30
Fe	34.60	3.61	12.29	45.17	125.72	34.21	27.23
Dibenzyline by Erythropoietin (D+Ep)							
No D or Ep	33.34	3.51	11.44	43.89	125.76	32.63	26.12
D	35.06	3.73	12.55	47.12	126.60	33.75	26.68
Ep	37.39	3.75	12.25	45.50	121.86	32.81	26.95
D+Ep	30.90	3.35	11.88	43.44	130.69	35.62	27.31

Table 3: continued.

Source (treatment)	Fetal Weights (gm.)	RBC (10^6/cmm.)	Hb (gm./100 ml.)	PCV (%)	MCV (cμ.)	MCH (μμg.)	MCHC (%)
Dibenzyline by Iron Dextran (D+Fe)							
No D or Fe	33.84	3.63	11.66	44.03	122.18	32.21	26.54
D	33.64	3.49	11.86	45.58	131.28	34.19	26.06
Fe	36.89	3.63	12.02	45.36	125.44	33.23	26.52
D+Fe	32.31	3.59	12.56	44.98	126.00	35.19	27.94
Erythropoietin by Iron Dextran (Ep+Fe)							
No Ep or Fe	33.40	3.66	12.19	46.78	128.37	33.32	26.11
Ep	34.08	3.46	11.34	42.82	125.09	33.07	26.49
Fe	34.99	3.59	11.80	44.23	123.98	33.06	26.69
Ep+Fe	34.21	3.64	12.78	46.11	127.46	35.36	27.77
Dibenzyline by Erythropoietin by Iron Dextran (D+Ep+Fe)							
No D, Ep, or Fe	29.14	3.51	11.40	43.61	124.97	32.47	26.28
D	37.67	3.8	12.97	49.96	131.77	34.18	25.94
Ep	38.55	3.75	11.92	44.45	119.39	31.95	26.81
D+Ep	29.62	3.17	10.76	41.19	130.78	34.19	26.17
Fe	37.55	3.51	11.46	44.18	126.54	32.80	25.95
D+Fe	32.44	3.66	12.13	44.28	121.41	33.32	27.42
Ep+Fe	36.23	3.76	12.58	46.54	124.34	33.67	27.09
D+Ep+Fe	32.18	3.53	12.99	45.68	130.59	37.05	28.46

DISCUSSION

Erythropoietin, iron dextran, dibenzyline, or combinations of them were administered to doe rabbits in the last trimester of pregnancy to study their effect on the hemoglobin system of the does and fetuses.

Although hemogram value differences were not statistically significant for the does some treatment mean decreases, relative to controls, were consistent with the statistically significant increases found for the fetuses. Decreased mean doe MCH values in all groups following treatment and decreased doe MCHC values in all groups except for iron dextran plus dibenzyline and iron dextran plus dibenzyline plus erythropoietin treatments occurred. These decreases may indicate hemoglobin precursors are transferred to the fetuses. Possibly the iron dextran plus dibenzyline and iron dextran plus dibenzyline plus erythropoietin treatments increased hemoglobin precursor formation in the does sufficiently to compensate for transfers to fetuses. Placental transfer seemed to take precedence over maternal utilization.

Differences between treated fetuses were statistically significant in several parameters. Analysis of the weight data revealed that the dibenzyline effect was significant as well as its interaction with erythropoietin and iron dextran and the three factor (dibenzyline, erythropoietin, and iron dextran) interaction. A closer look at the treatment means indicated that in the absence of either erythropoietin or iron dextran, dibenzyline produced an increase in weight. However,

in the presence of either or both erythropoietin and iron dextran a reduction resulted in weight by adding dibenzyline. The greatest weight gain resulted from erythropoietin alone and the least weight gain from the control. The main effect and interaction treatment means appear in table 3.

The only significant effect on the red blood cell count was the dibenzyline times erythropoietin interaction which was highly significant. The packed cell volume values showed highly significant changes in the dibenzyline times erythropoietin and iron dextran times erythropoietin interactions as well as significant changes in the three factor interaction group. Highly significant changes in the hemoglobin values occurred in the iron dextran times erythropoietin and dibenzyline times erythropoietin interactions and in the three factor interaction. Iron dextran alone also changed the hemoglobin values significantly. Significant changes in the MCV values occurred in the two factor interactions of iron dextran and erythropoietin, and iron dextran and dibenzyline. Dibenzyline caused highly significant alterations in the MCH values and the iron dextran and erythropoietin interaction caused a significant change in MCH. Treatments of iron dextran and erythropoietin alone elicited highly significant changes in the MCHC values as did the two factor interaction of iron dextran and dibenzyline. Complete analysis of variance for the seven parameters studied appear in the appendix table 4.

The chemicals apparently altered the hemoglobin sytems of the treated does and fetuses. However, hemogram values are not all uniformly changed by treatment with a single chemical or the

various combinations of the chemicals. This effect seems consistent with results in the literature. Swanson[133] reported that swine partial protein-free filtrate (PPFF) plus iron dextran when administered prepartum to swine seemingly promoted iron transfer and incorporation into hemoglobin in one day old piglets. PPFF seemed to be transfered to the fetuses enhancing highest packed cell volume values at 49 days of age. Lippert[83] showed no significant enhancement of erythropoiesis employing L-ascorbic acid, iron dextran, and rabbit erythropoietin treatments prepartum in doe rabbits.

SUMMARY

Effects of administered erythropoietin, dibenzyline, and iron dextran upon the hemograms of Dutch Belted doe rabbits and fetuses were investigated. Hemoglobin concentration, packed cell volume, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration parameters were involved. The experimental animals were maintained on a pelletized rabbit diet.

The animals were divided into control and experimental groups. The experimental treatments were as follows: iron dextran; erythropoietin; dibenzyline; iron dextran and erythropoietin; dibenzyline and erythropoietin; iron dextran and dibenzyline; and iron dextran, dibenzyline, and erythropoietin. Pretreatment doe blood samples were taken on day 25 of gestation and posttreatment samples on day 30 of gestation, then fetuses were removed by cesarean section and fetal blood samples taken.

Statistical analysis of fetal and doe hematological data was performed using analyses of variance. Fetal data showed highly significant ($P < .01$) increases in hemoglobin for iron dextran administration. Iron administration possible caused placental transfer of hemoglobin precursors. Refer to table 3 for the average main effects and interaction values for iron dextran, dibenzyline, and erythropoietin treatments. Significant changes in fetal weight indicated the effect of dibenzyline was to decrease body weight.

However, in the absence of either erythropoietin or iron dextran dibenzyline caused a weight increase. Doe hematological data showed no significant changes, possible indicating preferential transfer of hemoglobin precursors.

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APPENDIX

Table 4: Analyses of Variance in Control and Experimental Fetuses.

Basis	Source (treatment)	d. f.	Sum of squares	Mean squares	"F"
Weight					
	Fe	1	17.01	17.01	NS
	Ep	1	0.07	0.07	NS
	D	1	131.88	131.88	7.07**
	FexEp	1	12.39	12.39	NS
	DxEp	1	388.51	388.51	20.82**
	FexD	1	110.41	110.41	5.92*
	FexDxEp	1	494.58	494.58	26.50**
	Error	86	1605.08	18.66	
Red Blood Cell					
	Fe	1	0.071	0.071	NS
	Ep	1	0.117	0.117	NS
	D	1	0.191	0.191	NS
	FexEp	1	0.379	0.379	NS
	DxEp	1	2.255	2.255	13.92**
	FexD	1	0.061	0.061	NS
	FexDxEp	1	0.324	0.324	NS
	Error	86	13.929	0.162	
Packed Cell Volume					
	Fe	1	3.11	3.11	NS
	Ep	1	24.97	24.97	NS
	D	1	7.83	7.83	NS
	FexEp	1	196.99	196.99	9.91**
	DxEp	1	161.23	161.23	8.11**
	FexD	1	21.40	21.40	NS
	FexDxEp	1	108.33	108.33	5.45*
	Error	86	1709.87	19.88	

Table 4: continued.

Basis	Source (treatment)	d. f.	Sum of squares	Mean squares	"F"
Hemoglobin					
	Fe	1	6.44	6.44	5.32*
	Ep	1	0.11	0.11	NS
	D	1	3.19	3.19	NS
	FexEp	1	19.40	19.40	16.03**
	DxEp	1	12.75	12.75	10.54**
	FexD	1	0.67	0.67	NS
	FexDxEp	1	8.74	8.74	7.22**
	Error	86	109.14	1.21	
MCV					
	Fe	1	23.53	23.53	NS
	Ep	1	0.22	0.22	NS
	D	1	539.28	539.28	5.38*
	FexEp	1	264.92	264.92	NS
	DxEp	1	368.39	368.39	NS
	FexD	1	420.90	420.90	4.20*
	FexDxEp	1	66.34	66.34	NS
	Error	86	8612.77	100.15	
MCH					
	Fe	1	23.65	23.65	NS
	Ep	1	24.34	24.34	NS
	D	1	88.99	88.99	12.43**
	FexEp	1	37.69	37.69	5.26*
	DxEp	1	16.61	16.61	NS
	FexD	1	0.01	0.01	NS
	FexDxEp	1	7.84	7.84	NS
	Error	86	615.69	7.16	

Table 4: continued.

Basis	Source (treatment)	d. f.	Sum of squares	Mean squares	"F"
MCHC					
	Fe	1	19.95	19.95	15.23**
	Ep	1	12.34	12.34	9.42**
	D	1	5.00	5.00	NS
	FexEp	1	2.95	2.95	NS
	DxEp	1	0.24	0.24	NS
	FexD	1	20.98	20.98	16.02**
	FexDxEp	1	0.05	0.05	NS
	Error	86	112.99	1.31	

* $P < .05$
** $P < .01$
NS Not significant

Table 5: Hemogram Values of Saline Treated Does and Fetuses.

Animal	Weight (gm.)	RBC (10^6/cmm.)	Hb (gm./100ml.)	PCV (%)	MCV (cμ.)	MCH (μμgm.)	MCHC (%)
Doe 1 Initial-		4.96	11.8	37.8	76.1	23.8	31.3
Doe 1 Final -		4.31	10.1	30.0	69.6	23.4	33.7
Fetus	1 38	2.35	8.1	29.0	123.4	34.5	27.9
	2. 34	3.14	9.7	35.5	130.1	30.9	27.3
	3 35	2.50	8.4	32.0	128.0	33.6	26.3
	4 33	3.28	10.1	36.0	109.9	30.8	28.1
	5 32	3.32	10.4	37.0	111.4	31.3	28.1
Doe 2 Initial-		5.44	12.8	38.5	70.8	23.5	33.2
Doe 2 Final -		6.37	13.0	40.0	62.8	20.4	32.5
Fetus	1 22	3.76	12.8	51.0	135.8	34.0	25.0
	2 30	3.67	11.4	46.5	126.7	30.9	24.4
	3 24	3.89	12.6	49.0	126.1	32.4	25.7
	4 31	3.73	12.4	48.0	128.9	33.3	25.8
	5 25	3.77	11.8	45.5	120.8	31.3	25.9
	6 26	3.78	12.0	47.0	124.3	31.7	25.5
	7 21	4.03	13.3	51.0	126.6	33.1	26.1
	8 26	3.98	14.1	53.0	133.2	35.4	26.5
	9 31	4.02	12.6	50.0	124.4	31.3	25.2

Table 6: Hemogram Values of Iron Dextran Treated Does and Fetuses.

Animal	Weight (gm.)	RBC (10^6/cmm.)	Hb (gm./100ml.)	PCV (%)	MCV (cμ.)	MCH (μμgm.)	MCHC (%)
Doe 1 Initial-		6.32	13.4	40.0	63.3	21.2	33.5
Doe 1 Final -		4.48	11.0	34.0	75.9	24.6	32.4
Doe 2 Initial-		5.78	13.2	38.0	65.8	22.9	34.7
Doe 2 Final -		6.15	13.8	41.0	66.7	22.4	33.5
Fetus	1 34	3.79	12.5	45.0	118.7	33.0	27.8
	2 36	3,65	12.3	48.0	131.5	33.7	25.6
	3 32	3.92	11.8	43.0	109.7	30.1	27.4
	4 35	3.88	12.0	47.5	122.4	30.9	25.3
	5 36	3.62	12.1	46.0	127.1	33.4	26.3
Doe 3 Initial-		2.12	6.0	18.8	88.4	28.3	31.6
Doe 3 Final -		4.62	10.3	31.5	68.2	22.3	32.7
Fetus	1 40	2.80	10.4	40.0	142.9	37.1	26.0
	2 39	3.24	10.9	42.0	129.6	33.6	26.0
	3 38	3.57	11.8	45.0	126.1	33.1	26.2
	4 41	3.32	11.1	42.5	128.0	33.4	26.1
	5 40	3.49	10.7	44.0	126.1	30.7	24.3
	6 42	3.31	10.5	43.0	129.9	31.7	24.4

Table 7: Hemogram Values of Erythropoietin Treated Does and Fetuses.

Animal	Weight (gm.)	RBC (10^6/cmm.)	Hb (gm./100ml.)	PCV (%)	MCV (cμ.)	MCH (μμgm.)	MCHC (%)
Doe 1 Initial-		4.35	12.0	35.5	81.7	27.6	33.8
Doe 1 Final -		5.19	11.3	35.0	67.5	21.8	32.3
Doe 2 Initial-		4.93	11.0	33.0	74.8	22.3	33.3
Doe 2 Final -		4.49	9.8	32.0	71.3	21.8	30.6
Fetus	1 34	3.75	11.3	43.5	116.0	30.1	26.0
	2 35	3.96	12.0	47.5	120.1	30.3	25.3
	3 41	3.62	11.0	42.0	116.2	30.4	26.2
	4 36	3.59	11.2	42.5	118.6	31.1	26.2
	5 42	4.25	12.3	47.0	110.6	28.9	26.1
	6 33	3.99	11.6	44.0	110.3	29.1	26.4
Doe 3 Initial-		3.49	9.2	28.0	80.3	26.4	32.9
Doe 3 Final -		5.42	10.8	35.0	64.6	19.9	30.8
Fetus	1 40	3.11	12.6	48.0	154.3	40.5	26.3
	2 38	3.68	12.3	46.0	125.2	33.4	26.7
	3 40	4.01	13.2	47.0	117.2	32.8	28.0
	4 42	3.46	11.2	40.5	117.2	32.4	27.7
	5 43	3.81	12.4	41.0	107.6	32.4	30.1

Table 8: Hemogram Values of Dibenzyline Treated Does and Fetuses.

Animal	Weight (gm.)	RBC (10^6/cmm.)	Hb (gm./100ml.)	PCV (%)	MCV (cμ.)	MCH (μμgm.)	MCHC (%)
Doe 1 Initial-		5.70	12.7	38.8	68.0	22.3	32.8
Doe 1 Final -		-	-	-	-	-	-
Doe 2 Initial-		5.43	13.3	38.0	70.0	24.5	35.0
Doe 2 Final -		5.71	11.3	37.5	65.7	19.8	30.1
Fetus	1 40	3.46	11.5	47.0	136.0	33.3	24.5
	2 38	4.01	12.0	49.0	122.2	30.0	24.5
	3 42	3.89	12.5	45.5	117.0	32.1	27,5
	4 40	3.98	13.0	51.0	128.1	32.7	25.5
	5 43	3.80	12.8	50.0	131.9	33.8	25.6
	6 39	3.66	11.9	48.5	132.9	32.6	24.5
	7 41	3.89	12.0	46.5	119.8	30.9	25.8
Doe 3 Initial-		4.84	11.0	33.8	69.7	22.7	32.6
Doe 3 Final -		3.38	8.1	24.0	71.1	23.9	33.6
Fetus	1 37	3.82	13.8	50.0	130.9	36.0	27.5
	2 33	4.15	13.7	51.5	124.2	33.1	26.6
	3 32	3.54	14.6	56.0	158.2	41.2	26.1
	4 34	3.73	13.7	51.0	136.7	36.7	26.9
	5 33	3.74	14.1	53.5	143.2	37.8	26.4

Table 9: Hemogram Values of Iron Dextran and Erythropoietin Treated Does and Fetuses.

Animal		Weight (gm.)	RBC (10^6/cmm.)	Hb (gm./100ml.)	PCV (%)	MCV (cμ.)	MCH (μμgm.)	MCHC (%)
Doe 1	Initial-		4.42	10.0	29.0	65.6	22.6	34.5
Doe 1	Final -		4.75	10.4	30.5	64.2	21.9	34.1
Fetus	1	34	3.49	11.6	43.5	124.8	33.3	26.7
	2	38	3.59	12.5	46.0	128.1	34.7	27.1
	3	34	3.24	11.5	42.0	129.8	35.5	27.4
	4	32	3.42	12.4	46.5	136.0	36.1	26.6
	5	34	3.18	10.7	38.0	119.5	33.6	28.2
	6	30	4.19	13.6	50.0	119.3	32.5	27.2
	7	36	3.20	12.1	41.0	128.1	37.8	29.5
Doe 2	Initial-		3.13	9.0	22.0	70.4	28.8	40.9
Doe 2	Final -		5.56	11.7	38.0	68.3	21.0	30.7
Fetus	1	41	3.80	11.6	42.0	110.5	30.5	27.6
	2	38	4.39	12.7	47.0	107.1	28.9	27.0
Doe 3	Initial-		4.23	9.7	32.0	75.7	22.9	30.3
Doe 3	Final -		4.38	10.8	33.0	75.3	24.5	32.6
Fetus	1	41	3.46	13.0	49.0	141.6	37.6	26.5
	2	38	4.15	13.3	50.0	120.6	32.2	26.7
	3	34	4.18	13.7	53.0	126.8	32.8	25.8
	4	41	4.60	14.8	57.0	124.0	32.2	26.0

Table 10: Hemogram Values of Erythropoietin and Dibenzyline Treated Does and Fetuses.

Animal	Weight (gm.)	RBC (10^6/cmm.)	Hb (gm./100ml.)	PCV (%)	MCV (cμ.)	MCH (μμgm.)	MCHC (%)
Doe 1 Initial-		5.20	12.6	36.8	70.7	24.2	34.3
Doe 1 Final -		5.69	12.0	38.0	66.8	21.1	31.6
Doe 2 Initial-		5.62	11.3	34.8	61.9	20.1	32.5
Doe 2 Final -		5.55	11.0	35.0	63.1	19.8	31.4
Fetus	1 29	3.52	10.7	42.0	119.3	30.4	25.5
	2 30	3.24	10.7	42.5	131.4	33.1	24.2
	3 29	3.82	11.8	45.0	118.0	30.9	26.2
	4 30	3.20	10.4	40.0	125.2	32.6	26.0
	5 31	3.23	11.3	44.0	136.4	35.0	25.7
	6 26	3.23	11.0	44.0	136.4	34.1	25.0
Doe 3 Initial-		5.31	11.3	34.3	64.5	21.3	33.0
Doe 3 Final -		4.85	9.8	31.5	64.9	20.2	31.1
Fetus	1 24	2.23	10.1	37.0	159.8	43.6	27.3
	2 29	3.09	11.0	41.5	134.5	35.5	26.9
	3 33	2.95	10.1	39.0	132.4	34.3	25.9
	4 27	3.00	10.2	39.0	130.0	34.0	26.2
	5 35	3.11	10.7	40.0	128.6	34.4	26.8
	6 31	3.47	11.5	42.5	122.7	33.1	27.0
	7 31	3.11	10.4	39.0	125.4	33.4	26.7

Table 11: Hemogram Values of Iron Dextran and Dibenzyline Treated Does and Fetuses.

Animal	Weight (gm.)	RBC (10^6/cmm.)	Hb (gm./100ml.)	PCV (%)	MCV (cμ.)	MCH (μμgm.)	MCHC (%)
Doe 1 Initial-		5.18	12.5	37.5	72.4	24.1	33.3
Doe 1 Final -		6.77	12.9	40.5	59.9	19.0	31.9
Fetus	1 38	4.02	11.8	45.0	112.1	29.4	26.3
	2 30	4.21	13.0	49.0	116.5	31.5	26.5
Doe 2 Initial-		5.32	11.2	36.0	67.7	21.0	31.0
Doe 2 Final -		5.55	10.6	23.0	41.4	19.1	46.1
Fetus	1 30	3.28	12.3	43.5	132.8	37.6	28.3
	2 22	4.24	14.1	51.0	120.3	33.2	27.6
	3 26	3.87	13.3	49.0	126.6	34.4	27.1
	4 28	3.82	12.0	43.0	112.6	31.4	27.9
	5 30	3.39	11.0	40.0	118.0	32.5	27.6
Doe 3 Initial-		4.63	12.0	35.5	76.7	25.9	33.8
Doe 3 Final -		5.42	12.1	38.0	70.1	22.3	31.8
Fetus	1 46	3.15	10.7	38.5	122.4	34.0	27.8
	2 42	3.01	11.0	39.5	131.4	36.4	27.7

Table 12: Hemogram Values of Iron Dextran, Dibenzyline, and Erythropoietin Treated Does and Fetuses.

Animal	Weight (gm.)	RBC (10^6/cmm.)	Hb (gm./100ml.)	PCV (%)	MCV (cμ.)	MCH (μμgm.)	MCHC (%)
Doe 1 Initial-		4.70	9.7	33.0	70.3	20.7	29.4
Doe 1 Final -		-	-	-	-	-	-
Doe 2 Initial-		5.54	11.6	36.5	65.9	20.9	31.8
Doe 2 Final -		5.12	11.0	34.5	67.4	21.5	31.9
Fetus	1 36	4.30	13.8	50.0	116.4	32.0	27.5
	2 32	3.67	12.9	46.5	126.9	35.2	27.7
	3 32	3.43	12.2	43.5	127.0	35.6	28.0
	4 26	2.81	11.7	43.0	153.0	41.6	27.2
	5 28	3.26	12.0	42.0	128.8	36.8	28.6
	6 30	3.37	13.0	41.5	123.1	38.5	31.3
	7 32	3.10	12.6	47.5	153.2	40.6	26.5
	8 32	3.69	13.2	48.5	131.4	35.8	27.2
Doe 3 Initial-		6.03	13.8	41.0	68.0	22.9	33.6
Doe 3 Final -		6.53	13.0	40.0	61.3	19.9	32.5
Fetus	1 32	3.98	15.5	48.0	120.6	38,9	32.3
	2 38	3.65	13.0	47.0	128.8	35.6	27.7
	3 36	3.54	13.0	45.0	127.2	36.7	28.9